REMARKS

This paper is responsive to the Final Office Action mailed on January 5, 2006. Claims 1-19, 22-30 and 32-36 are pending in the present application. Applicants are pleased to note that Claims 1-16 and 19 are allowed. Claims 30, 35 and 36 were found to be allowable if rewritten to include all of the limitations of the claims from which they depend. These claims have been so amended and thus are believed to be in condition for allowance. No new matter is added by these amendments.

Claims 1 and 7 have been amended herein to replace the trade name "Neutrase" with the description of the underlying protease. Neutrase is well-known in the art to be a neutral protease derived from Bacillus amyloliquefaciens (see attached data sheet). Claim 5 has been amended to correct a typographical error. Applicants submit that these amendments also do not add new matter.

Claim Rejections Under 35 U.S.C. § 102

Claims 17, 18, 22, 23, 27-29 and 32-34 were rejected under 35 U.S.C. § 102(b) as being anticipated by Ju et al. (J. Dairy Sci. 78:2119-2128 (1995)). The Examiner found that Ju discloses the hydrolysis of whey protein isolate (WPI) with the claimed enzyme at pH 7.0 and 40°C, with the reaction being stopped by dilution and pH change to 2.5. Despite Applicants' previous amendment specifying an enzyme to substrate ratio of 0.1% to 3% (versus 10% as described by Ju) and Applicants' related arguments, the Examiner was not convinced that Ju would produce a different WPI hydrolysate. The Examiner asserted that because the process disclosed by the Applicants and the method disclosed by Ju involve the same starting material, enzyme, and achieve the same degree of hydrolysis, the same product must inherently be produced.

Applicants disagree and submit that Ju does not inherently anticipate the cited claims. In support of this position, the Declaration of Julian Robert Reid and Sophia Stathopolous under 37 C.F.R. §1.132, is attached. The Declaration reports the results of an actual comparison of the peptide profiles of WPI hydrolysates prepared by the method of Ju and by Applicants' process, as disclosed in Example 1 of the specification. Although the same degree of hydrolysis (DH) was reached in each process, the peptide profile of the hydrolysate product obtained by the method of

Ju is distinctly different from that of the product obtained Applicants' process. In other words, each hydrolysate product contains a number of peptides not found in the other sample. Thus, the results of the study detailed in the Declaration demonstrate that the product of Ju does not inherently anticipate the claimed products and compositions.

In view of the lack of inherent anticipation, Applicants respectfully request the withdrawal of the rejections under 35 U.S.C. §102.

Claim Rejections Under 35 U.S.C. § 103

Claims 17, 18, 22-29 and 32-34 were also rejected under 35 U.S.C. 103 (a) as being unpatentable over the combination of Schlothauer et al. (WO 99/65326) in view of Ju et al. (J. Dairy Sci. 78:2119-2128 (1995)).

Claims 17, 18, 22-29 and 32-34 are directed towards compositions and products prepared by the process of claim 1 and/or comprising one or more bioactive peptides produced by the process of claim 1. Claim 1 has been allowed. Applicants have also demonstrated that the peptide profile of the WPI hydrolysate produced by Applicants' process is distinct from the hydrolysate produced by the method of Ju, as detailed in the attached Declaration. Thus, as the process of claim 1 (from which each of the currently rejected claims ultimately depends) is not anticipated by or obvious in view of the cited references and the cited references do not inherently (or explicitly) teach the products of the process, Applicants submit that the claimed compositions and products can not be obvious and request withdrawal of the rejection under 35 U.S.C. §103.

In addition, Applicants note that in making the rejection the Examiner found that while Schlothauer's method is different from the present invention as it does not disclose the use of WPI as the starting material in the hydrolysis process, the statement that a "whey protein containing substrate" can be used in their process would motivate the skilled artisan to use WPI, as Ju teaches that WPI is a whey protein containing substrate. Applicants respectfully disagree with this assessment. Schlothauer is concerned with the production of bioactive peptides. As discussed in Applicants' previous response, it is common knowledge that WPC contains casein peptides that are biologically active. These casein peptides are lacking in WPI and as a result a hydrolysate of WIP would not have been expected to have the biological activity of a WPC

hydrolysate. . Thus, the knowledge in the art taught away from the subsitution of WPI for WPC in Schlothauer and a skilled artisan would not have been motivated to use WPI, as disclosed by Ju, in Schlothauer's process, since a WPC hydrolysate product would have been expected to provide more bioactive peptides than a WPI hydrolysate.

Further, Ju is directed to the gelling properties of WPI hydrolysates and has no teaching or suggestion of creating hydrolysates with bioactivity. Thus, Ju also fails to provide any motivation for the combination cited by the Examiner. In view of the lack of motivation to replace WPC with WPI in the process of Schlothauer, Applicants submit that a prima facie case of obviousness has not been established.

Finally, the WPI hydrolysate of the present invention was superior over the WPC hydrolysate of Schlothauer in terms of its bioactivity, flavour and functionality. The Examiner recognized the significance of these unexpected results in his reasons for allowing the process claims, but maintained the rejection of the product claims. Applicants submit that the unexpected nature of the results is equally applicable to all of the product claims and further supports the conclusion that the claims are not obvious over the combination of Schlothauer and Ju.

Conclusion

In view of the amendments and arguments presented above, Applicants submit that the present Application is in condition for allowance and respectfully request the same. If any issues remain, the Examiner is invited to contact Applicants' representative at the number provided below in order to resolve such issues promptly.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: June 30, 2006

By: _____

Andrew N. Merickel
Registration No. 53,317
Attorney of Record
Customer No. 20,995
(415) 954-4114

Please Direct All Correspondence to Customer Number **20995**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Schlothauer et al.
App. No	:	10/070,406
Filed	:	October 7, 2002
For	:	BIOACTIVE WHEY PROTEIN HYDROLYSATE
Examiner	:	Francisco Chandler Prats
Art Unit	:	1651

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

June 30, 2006
(Date)

Andrew N. Merickel, Reg. No. 53,317

DECLARATION OF JULIAN ROBERT REID AND SOPHIA STATHOPOLOUS UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

We, Julian Robert Reid and Sophia Stathopoulos, declare as follows:

1. We are current employees at Fonterra Co-operative Group Limited, located on Dairy Farm Road, Palmerston North, New Zealand.

2. Julian Robert Reid is a co-inventor of the above-captioned application ("the present application").

3. We understand that Ju et al. (J. Dairy Sci. 78:2119-2128 (1995)) has been cited against the present application in the Office Action mailed on January 5, 2006. We have reviewed Ju et al. and the Office Action.

4. This Declaration is submitted to show that the peptide profile of the WPI hydrolysate produced by Ju et al. is not the same as the peptide profile of the WPI hydrolysate claimed in the present application.

5. We conducted an experiment comparing the peptide profiles of the WPI hydrolysates prepared by the method of Ju et al. and by the method of the present application. This experiment was performed on March 31, 2006.

6. A particular aim of our experiment was to test whether or not "... the peptide profile will remain the same at any given degree of hydrolysis" for the hydrolysates produced according to the method either of Ju et al. and the present application, as asserted by the Examiner (see Office Action at page 4).

7. The enzyme Neutrase (sourced from *Bacillus subtilis*), was purchased from Novo Nordisk, Denmark. A895 Whey Protein Isolate (WPI) was prepared by Fonterra, New Zealand (92% protein).

8. The follow procedure to prepare WPI hydrolysate according to the method of Ju et al. was conducted. A895 WPI was reconstituted with water in a stirred stainless steel beaker at 12% w/w total protein (13.04g A895 WPI made up to 100.07g with Milli Q water), and stored overnight at 5°C. The following day the solution was placed in a 40°C water bath. Enzyme (Neutrase) was added to give an enzyme:substrate (E:S) ratio of 1:10. To prepare the enzyme for the reaction, Neutrase was first dissolved in water and the solution was allowed to stand for 10 minutes. The reaction was then initiated by adding the prepared enzyme to the reconstituted A895 WPI solution to give a final E:S ratio of 1:10. A895 WPI was hydrolysed for 3 hours at 40°C with gentle agitation. Throughout the reaction, samples were collected at 10-minute intervals, inactivated and analysed for degree of hydrolysis. Inactivation was performed by cooling the sample to 2°C for 2 minutes followed by heat treatment at 80°C for 20 minutes. The degree of hydrolysis was monitored using the MOPA assay, which is detailed in Appendix 1.

9. A WPI hydrolysate was also prepared according to the methods of the present application. Details of the method used to prepare the WPI hydrolysate are shown in Appendix 3.

10. Analysis of hydrolysates was performed using the reverse phase HPLC method described in Appendix 2.

11. The hydrolysate prepared by the method of the present application gave a degree of hydrolysis (DH) of 4.0%. The 160-minute hydrolysis sample produced by the method of Ju et al. also gave a DH of 4.0%. All samples were analysed in triplicate. The profiles obtained from each sample of the triplicate were identical. Therefore, one representative chromatogram was chosen from each.

12. The overlayed peptide profiles of hydrolysate samples with a DH of 4% produced by the two different methods are shown in Figure 1. It is apparent that although the two profiles have many similarities, there are also a number of significant significant quantitative and qualitative differences. Figure 2 shows the same overlayed chromatograms as Figure 1, but with the vertical axis (absorbance) reduced to highlight the differences. Figure 3 shows the peptide profile of the 160 minute sample obtained from the method of Ju et al. overlayed on a control peptide profile (WPI subjected to the method of Ju et al. except that no enzyme was added). The lack of peptide peaks in the control sample shows that there was no endogenous enzyme activity in the WPI substrate used.

13. Careful examination of the peptide profiles of the two hydrolysates reveals that, as expected, many of the peaks present in each sample share identical elution times, albeit with sometimes very different peak sizes. This indicates that the peptides responsible for such peaks are probably identical.

14. However, from Figure 2 it is clear that there are a number of peaks present in the hydrolysate prepared according to the present application that are not present in the hydrolysate prepared according to Ju et al. and vice versa. This is conclusive evidence that each hydrolysate

contained a number of peptides not found in the other sample. The peaks in Figure 2 that elute around 35 – 40 minutes (marked with blue arrows above the peaks) are a clear example (among others) of peptides that are unique to a hydrolysate produced by the methods of the present invention. Conversely, the five red arrows in Figure 2 (shown below the peaks) mark peptides that are unique to the hydrolysate of Ju et al.

15. The results of this study conclusively show that at any given DH, the method of Ju et al. does not produce the same set of peptides as does the method of the present application.

16. We further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 22 May 2006 By: _____
Julian Robert Reid

Dated: 22/05/06. By: _____
DD/MM/YY
Sophia Stathopoulos

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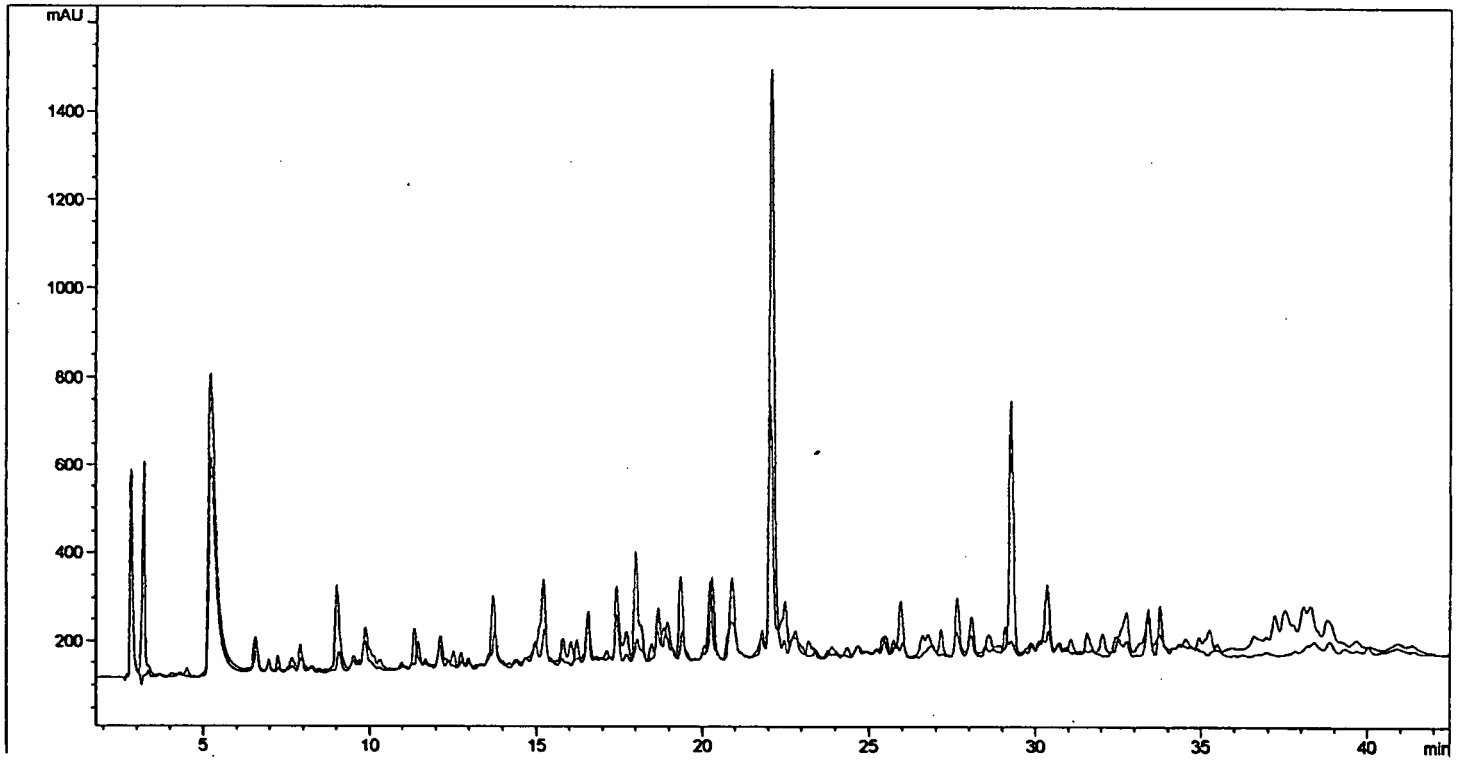

Figure 1. Overlay of the reverse phase HPLC chromatograms obtained from the hydrolysate produced using the method of Ju *et al.* (1995; shown as the red trace) and the hydrolysate produced using the method of the Schlothauer *et al.* (shown as the blue trace). The horizontal axis shows the HPLC run time and the vertical axis shows the absorption at 214 nm.

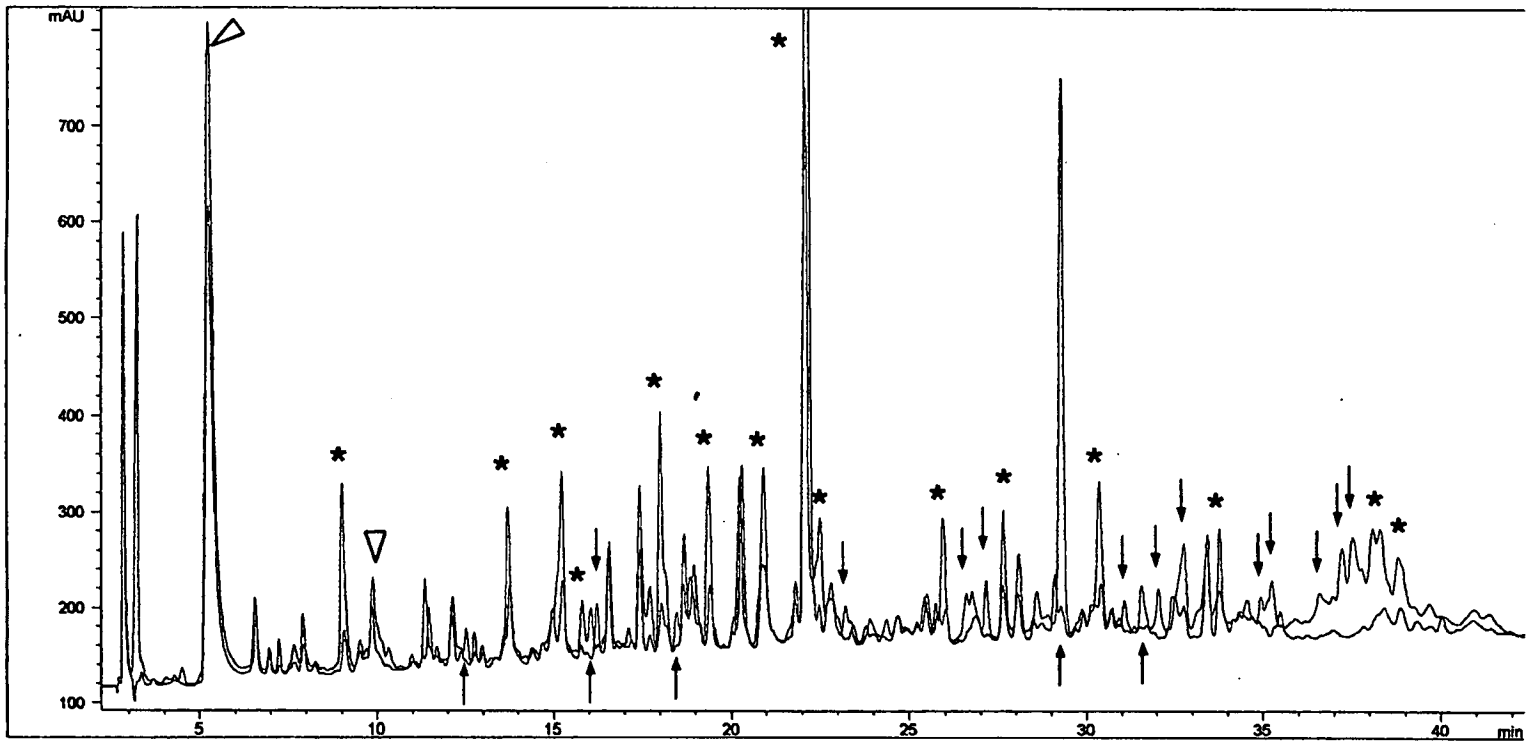

Figure 2. The same hydrolysate chromatograms from Fig. 1 are shown (red trace = hydrolysate of Ju *et al.* [1995]; blue trace = hydrolysate of the present invention – Schlothauer *et al*) with the scale of the vertical axis reduced to clearly illustrate differences in the peptide profiles. The various markings are: Δ, peaks resulting from the use of urea to solubilise the samples; *, peaks where clear quantitative differences are apparent; ↑, peaks present in the hydrolysate of Ju *et al.*, but not in the hydrolysate of the present invention; ↓, peaks present in the hydrolysate of the present invention, but not present in the hydrolysate of Ju *et al.*
The horizontal axis shows the HPLC run time and the vertical axis shows the absorption at 214 nm.

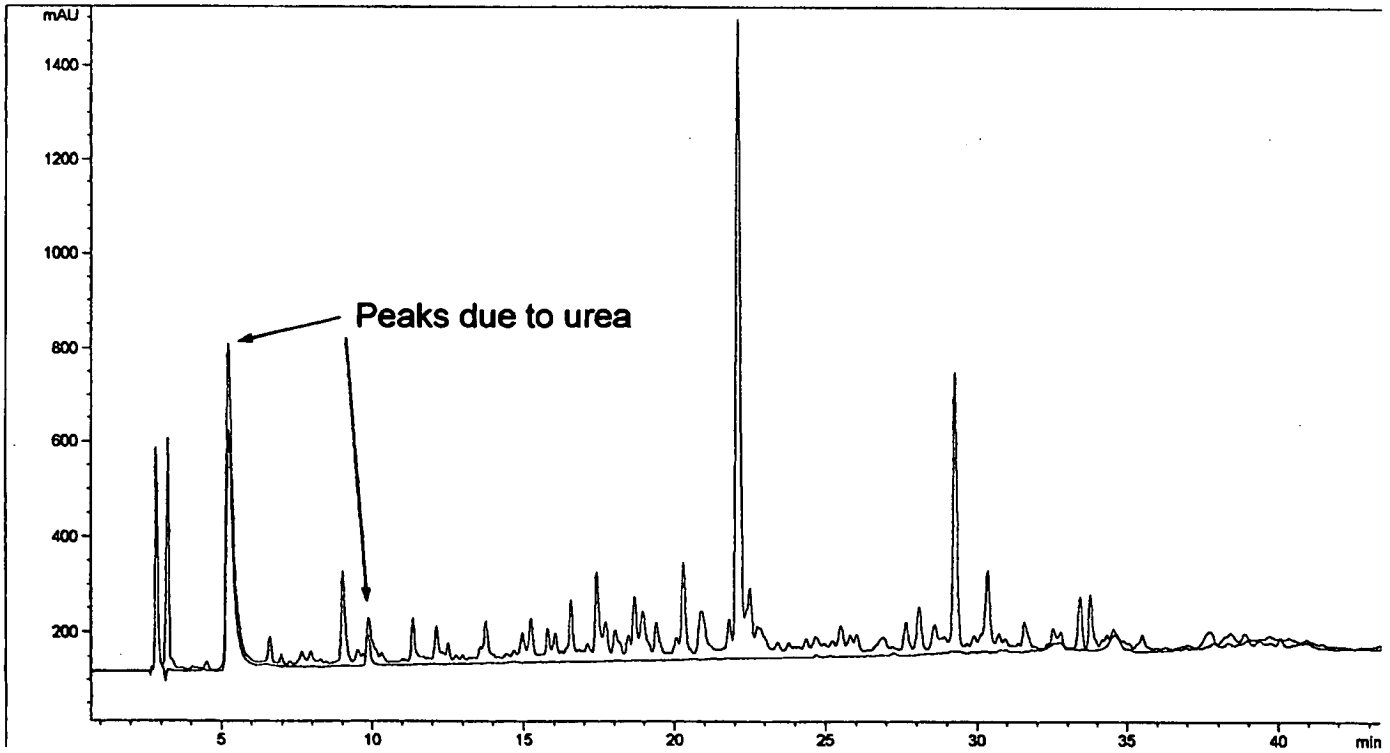

Figure 3. Overlay of the chromatogram obtained from reverse phase HPLC analysis of the hydrolysate produced using the method of Ju *et al.* (shown as red trace) and the chromatogram produced from the control sample i.e. WPI treated according to the method of Ju *et al.* in all respects except that no enzyme was added (shown as blue trace).
The horizontal axis shows the HPLC run time and the vertical axis shows the absorption at 214 nm.

Appendix 1

Degree of Hydrolysis by the Modified O-phthaldialdehyde (MOPA) Method

Reference: Frister H, Meisel H & Schlimme E (1988)
Freesenius Z Anal Chem. 330, 631-633

Principle: This method is used to determine the degree of hydrolysis (DH) of hydrolysed protein products. The sample is diluted in a MOPA buffer and the absorbance converted to µmol of amino groups by a glycine standard curve at 340nm.

Apparatus: Spectrophotometer operating at 340nm.

Materials and Reagents:

Reagents

- Di-sodium tetraborate (MW 381.36)
- Sodium dodecyl sulfate (SDS) (MW 288.4)**
- 2-(Dimethylamino) ethanethiol hydrochloride (MW 141.67)**
- O-phthaldialdehyde (MW 134.1)**
- Glycine (MW 75.1)
- Methanol (Analar)
- Milli-Q water

****Exercise care when handling (gloves and face mask must be worn)**

MOPA buffer

	%
250mL of 0.1M (38.136 g/l) di-sodium tetraborate	50.0
50 mL of 10% sodium dodecyl sulfate (SDS)	5.0
1g of 2-(Dimethylamino) ethanethiol hydrochloride	0.2

Mix above ingredients and bring to 500mL total volume with MilliQ water.
Store buffer in refrigerator. The buffer has limited shelf-life and should be used within a period of ten days.

Redissolve the SDS by gentle warming prior to use.

O-phthaldialdehyde (OPA) reagent

Dissolve 40 mg of OPA reagent in 1 mL of methanol, then make up to 50 mL total volume with MOPA buffer. 3 mL of OPA reagent is required for each sample and each standard assayed.
This reagent should be made fresh daily. Make just before use as it is affected by light.

Standard curve

1. Dissolve 3.0028 g/l of glycine in MilliQ water to make a 0.04 M solution and store in a refrigerator.
2. Dilute 1:10 in MOPA buffer to make 0.004 M solution (0.2 mL of 0.04 M glycine solution and 1.8 mL of MOPA buffer gives sufficient for the standard curve dilutions).
3. Prepare the following dilution series:

0.004 M glycine (mL)	MOPA buffer (mL)	µmol/200µL glycine
0.0625		0.05
0.125	0.875	0.10
0.1875	0.8125	0.15
0.25	0.75	0.20
0.375	0.625	0.30

Sample preparation

Dilute the samples in MOPA buffer to give a final concentration between 0.3 - 0.5mg/mL approx and mix well. Use 1mg/mL for lightly hydrolysed samples.
For powdered samples, weigh 3-5mg and add 10 mL of MOPA buffer. Record the exact weight of powder in g. For liquid samples record the dilution factor.

Procedure

1. Blank the spectrophotometer on OPA reagent.
2. Add 200µL of sample to cuvette and then add 1.5 mL of OPA reagent.
3. Read the absorbance at 340 nm after exactly 2 minutes.
4. Test the standards and samples in duplicate.

Calculations

1. Average all duplicate absorbances of samples and standards.
2. Plot µmol amino groups (glycine) versus absorbance for the standard curve.
3. Check the linearity of the standard curve and perform linear regression on the points in the linear range. Obtain the regression equation of the curve in the form:

Absorbance = (A*µmol amino groups) + B

Where: A is the slope of the curve
B is the constant

* multiplication

Note: the curve is usually linear throughout the dilution series.

4. Convert the absorbances of the samples to µmol amino groups (C). Check that the absorbances are in the linear range

$C = (Absorbance_{sample} - B)/A$

5. Calculate the weight of protein per sample in g in the OPA reaction mixture (D)

D = (wt sample/10)*0.2*%protein in sample/100

6. Calculate the µmol amino groups /g protein (H) for each sample
H = C/D

7. Calculate the degree of hydrolysis (DH) by the following formula. The formula subtracts the theoretical contribution of lysine side-chains (E) and expresses the remaining amino groups as a percentage of the total theoretical number of peptide bonds/g protein (H_{total})

$DH = (H - E)/ H_{total}*100$

E and H_{total} are defined for each substrate in the table below.

Protein substrate	**H_{total} (µmol NH_2/g protein)**	**E (µmol NH_2/g protein)**
Casein	8200	560
Acid wpc	8800	650
Cheese/rennet wpc	8800	570
Lactalbumin	8800	730
TMP	8320	578

Appendix 2

Reverse phase chromatography

Samples inactivated at 80°C for 20 min were dissolved by adding urea, mercoptoethanol, SDS to final concentrations of 3M, 35mM and 0.5% (w/v), respectively. TFA was added to a final concentration of 1% (v/v). Prior to analysis samples were centrifuged and supernatant was used for analysis.

Samples were analysed using a Hewlett Packard series 1100 HPLC. Samples (10 μL) were loaded onto a protein and peptide C18 GRACEVYDAC column [218TP54, 5micron particle size, 300 Å pore size] and eluted using a gradient of 0-50% solvent B in 50 min (Solvent A = 0.1% TFA in water, Solvent B = 0.08% TFA in acetonitrile) and a flow rate of 1 mL/min. Absorption of eluate was monitored at 214 nm.

Appendix 3

Example 1

Pilot plant production of WPI mild hydrolysate

Whey protein isolate produced by cation ion exchange technology (ALACEN™ 895) with a protein content ≥ 90% w/w was reconstituted at 20% total solids in water (50°C) Reconstituted ALACEN™ 895 was transferred to a 150L tank at 50°C. Water (50°C) was added to the tank to make final total solids of 4%. The solution was stirred and Neutrase (E:S 0.9%) was added.

Two hours after enzyme addition the first hydrolysate was pumped through the UHT plant. Enzyme inactivation was achieved using direct steam injection to heat the hydrolysate to 88°C and the hydrolysate was held it at this temperature for 1.5 seconds. The hydrolysate was flash cooled and passed through shell and tube heat exchanges to cool to ambient temperature.

The hydrolysate was subsequently evaporated and dried.

A hydrolysate made following the process of Example 1 had the following features:

Solubility: 95%
Heat stability: 120°C for 10 min @ 5% TS solubility 95%
ACE-I in vitro activity: 289 mg/L IC50
Foaming: Markedly increased over non-hydrolysed WPI
Flavour: Markedly improved over WPC based mild hydrolysates
Appearance: opaque white, particle size ~0.1 µm

DK

Neutrase®

Description

Neutrase is an endo-protease which can be used in most cases where proteins have to be broken down either moderately or more extensively to peptides.

Neutrase is a bacterial protease produced by a selected strain of *Bacillus amyloliquefaciens*.

Neutrase contains only the neutral part of *B. amyloliquefaciens* proteases, whereas most other commercial preparations also contain the alkaline protease.

Neutrase is a metallo protease (Zn), which is stabilized with Ca^{++} and consequently inhibited by EDTA.

Neutrase contains a non-standardized amount of beta-glucanase and is free of any alpha-amylase activity.

Applications

Neutrase is used to upgrade proteins of vegetable and animal origin.

Detailed recommendations with respect to applications are given in separate papers which are available on request.

Activity

Neutrase is available in the following standard strengths:

Liquid:	Neutrase 0.5 L	0.5 AU/g
Granulate:	Neutrase 1.5 MG	1.5 AU/g

The products are standardized in Anson Units (AU). The analytical method, AF 4, which is based on denatured hemoglobin in a 0.02 M Ca^{++} buffer, is available on request.

Product Types

Neutrase 0.5 L is a clear brown liquid with a density of approx. 1.25 g/ml.

Neutrase 1.5 MG is a light brown, free flowing, non-dusting micro-granulate with an average particle size of approx. 300 microns.

Product Specification

Neutrase complies with FAO/WHO JECFA and FCC recommended specifications for food grade enzymes, supplemented with maximum limits of 5×10^4/g for total viable count and 10^2/g for moulds.

Product Characteristics

The optimal working conditions for Neutrase are at 45-55>C and pH 5.5-7.5. The activities shown in Figures 1-3 are measured according to a modified Anson method in aqueous solutions without the stabilizing effect of proteinaceous matter.

The stability of Neutrase at a certain temperature is influenced by the type and concentration of the proteins present.

Examples of stability expressed as decimation time (t_0) at different temperatures at pH 7 and 8% protein concentration for different substrates are shown in Figure 4 t_0 is the time required to reduce the activity to 1/10. Inactivation is obtained at $3 \times t_0$. Addition of Ca^{++} to these natural substrates does not influence the stability of Neutrase.

Neutrase can be inactivated by heat treatment, e.g. 2 minutes at 85°C.

Packing

Neutrase 0.5 L is available in jerry cans containing 30 kg or in steel drums with 250 kg.

Neutrase 1.5 MG is supplied in 60-litre drums containing 40 kg.

Novo Nordisk

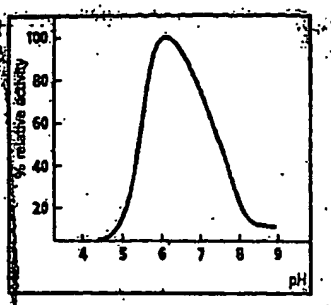

Fig. 1. The influence of pH on the activity of Neutrase at 45°C.

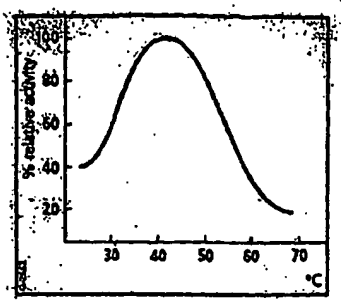

Fig. 2. The influence of temperature on the activity of Neutrase at pH 6.0.

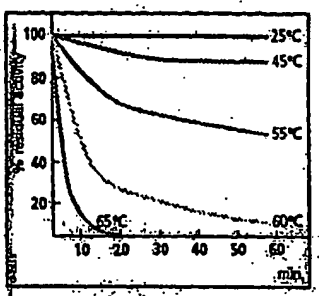

Fig. 3. The stabilities at pH 6.0 (phosphate buffer) of Neutrase at various temperatures.

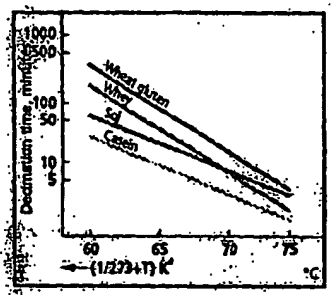

Fig. 4. Inactivation of Neutrase: 6 at pH 7 and 8% protein.

Solubility

The active components of Neutrase are readily soluble in water at all concentrations that occur in normal usage.

Handling Precautions

Neutrase is formulated in a way that gives the highest degree of safety during handling.

The product is non-flammable, completely miscible with water, and safe when used according to directions. Proteolytic enzymes may irritate skin or eyes, and enzyme dust may cause sensitization when inhaled.

Observe standard handling precautions to avoid direct contact with the product or inhalation of dust from the dried product. In case of spillage and accidental contact with the skin or eyes, rinse promptly with water.

Separate leaflets, "How to handle liquid Novo Nordisk enzymes safely" and "How to handle powder/granulated Novo Nordisk enzymes - safely", are available on request.

Storage

When stored at 5°C, the products will maintain the declared activity for at least 1 year. When stored at 25°C, the products will maintain the declared activity for at least 3 months.

Neutrase 0.5 L should be stored at temperatures above -10°C.

Neutrase 1.5 MG should be stored in a dry place and at constant temperature in order to avoid condensation of water inside the drum, which could accelerate the activity decrease.

Enzyme Business

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B 213h-GB 3000 April 1996 © Novo Nordisk A/S

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